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BROWNING PREVENTION METHOD FOR ASCORBIC ACID

Takeo Inoue and Kayo Akiyama

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ASCORBIC ACID BROWNING PREVENTION METHOD

[Asukorubinsan no katsuhen boshi hoho]

Inventors: Takeo Inoue
Kayo Akiyama

Applicant: Sanei Chemical Corporation

[There are no amendments to this patent.]

Claims

1. A browning prevention method for ascorbic acid or a derivative thereof, characterized in that a flavonoid glycoside is mixed into ascorbic acid or a derivative thereof.

2. The method described in Claim 1, in which the flavonoid glycoside is 1, or a mixture of 2 or more, of rutin, quercetrin, isoquercetin, peltatoside, or hyperoside.

3. The method described in Claim 1, in which the flavonoid is a water-soluble flavonoid glycoside obtained by causing an enzyme that has a glycosyl transfer action to act on 1, or a

mixture of 2 or more, of rutin, quercetrin, isoquercetin, peltatoside or hyperoside in the presence of lactose or a galacto-oligosaccharide and/or starch.

4. The method described in Claim 3, in which the enzyme that has a glycosyl transfer action is an enzyme that has a galactose residual group transfer action or an enzyme that has a glucose residual group transfer action, or a mixture of an enzyme that has a galactose residual group transfer action and an enzyme that has a glucose residual group transfer action.

Detailed explanation of the invention

Industrial application field

The present invention relates to a browning prevention method for ascorbic acid or a derivative thereof. Therefore, the fields of application are the pharmaceutical industry, the cosmetics industry, and the food industry in which ascorbic acid and derivatives thereof are used.

Prior art

Ascorbic acid has been widely used in the pharmaceutical field as vitamin C since being isolated by Szent-Györgyi et al. around 1930 as a factor against scurvy. It is known that ascorbic acid is not limited only to preventing scurvy but has many useful effects, e.g., increasing the activity of hormones and enzymes, suppressing the effects of harmful substances, improving the body's power of resistance, and the like. In the field of foods, too, its outstanding oxidation ability is also used to improve food preservation and maintain food quality as an antioxidant, a browning inhibitor, a meat coloring auxiliary and a color fade inhibitor.

However, the browning reaction due to auto-oxidation of the ascorbic acid itself is known for ascorbic acid and usage methods for it are limited. It is known that with auto-oxidation of ascorbic acid alone, generally the ascorbic acid passes through dehydroascorbic acid and diketogulonic acid, and decarboxylation and dehydration reactions are further repeated to produce a browned compound (for example, Nihon Shokuhin Kogyo Gakkaishi, Vol. 33, No. 6, pp. 456-462, 1986). In addition, acceleration of the browning reaction is also seen when amino acids are also present.

Problem to be solved by the invention

The present invention presents a method that has the effect of preventing the abovementioned browning reaction in ascorbic acid.

Means to solve the problem

The present inventors pursued assiduous research, and as a result, they have invented a method to prevent browning of ascorbic acid or derivatives thereof by using a flavonoid glycoside.

The present invention is accomplished by adding an equal quantity or less of flavonoid glycoside to ascorbic acid. The ascorbic acid used for the present invention may be, in addition to a free acid, a derivative of ascorbic acid, e.g., a salt of sodium or the like, an ester of a fatty acid or the like, or an ether of a saccharide or the like. The ascorbic acid concentration can be changed according to the targeted pharmaceutical form and cannot be determined definitively, but it is generally preferably at least 0.01% (wt%, same hereafter). The flavonoid glycosides used with the present invention include, in addition to rutin, quercetrin, isoquercetin, peltatoside and hyperoside, water-soluble flavonoid glycosides obtained by having an enzyme that has a galactose residual group transfer action or an enzyme that has a glucose residual group transfer action, or a mixture of an enzyme that has a galactose residual group transfer action and an enzyme that has a glucose residual group transfer action act on 1, or a mixture of 2 or more, of these glycosides. The flavonoid glycosides may be used alone or they may be used in mixtures of 2 or more. They may also be used in liquid form by dissolving in water, ethanol or another solvent, or a mixture of them, or they could be used in the form of a solid substance or powder. There is also no hindrance to using metaphosphoric acid, dicarboxylic acid, tricarboxylic acid, EDTA, phytic acid or other compounds that are generally considered to contribute to stabilization of ascorbic acid.

It is beneficial that the water-soluble flavonoid glycoside be produced using a method such as the water-soluble flavonol glycoside preparation method in Japanese Kokai Patent Application No. Hei 1[1989]-213293 previously filed by the applicant of this invention, the water-soluble flavonol glycoside preparation method in the patent application filed by the same applicant on July 6, 1990, the water-soluble glycoside [preparation method] in the patent application of July 6, 1990 by the same, the water-soluble flavonol glycoside preparation method in the patent application of July 6, 1990 by the same, or the flavonol glycoside reforming method in the patent application of July 6, 1990 by the same.

Reference Example 1

10 g of rutin were dispersed in 2 L of water, 1 g of naringinase preparation (made by Amano Seiyaku KK, trade name: "Amano" naringinase) was added, and it was kept at 60°C for 24 h. The pH of this system was 6. This was cooled to below 10°C, and 6 g of a precipitate composed of isoquercetin was obtained. 5 g of the precipitate and 30 g of cornstarch were added to 5 L of a 0.01 M disodium hydrogen phosphate-sodium dihydrogen phosphate buffer solution with pH of 6.7, this was homogenized, 2 mL of cyclodextrin glucanotransferase preparation (made by Amano Seiyaku KK, trade name: Contizyme) were added, and this was kept at 55°C for 2 h. This substance was concentrated and dried, and 36 g of a yellow solid was obtained (hereafter, the flavonoid glycoside obtained in Reference Example 1 is abbreviated glycoside A).

Reference Example 2

20 g of glycoside A prepared with the method of Reference Example 1 and 200 g of lactose were dissolved in 100 mL of 0.1M phosphoric acid buffer solution (pH 7.0). 1 g of β-galactosidase (enzyme activity value: 20,000 units) derived from Bacillus circulans made by Daiwa Kasei KK was added, and this was stirred for 4 h at 60°C. After reaction completion, the mixture was diluted with 1 L of water, it was passed for 1 hour into a column filled with 1000 mL of a porous polymer made of a styrene-divinyl benzene copolymer, and then 5 L of ion-exchanged water was passed for 1.5 hour. Next, 2 L of 40 v/v% methanol was passed for 1 hour and the adhered substances were dissolved out. The methanol solution was concentrated and dried, and 25 g of a yellow solid substance was obtained (hereafter, the flavonoid glycoside obtained in Reference Example 2 is abbreviated glycoside B).

The mechanism of action by flavonoid glycosides in preventing browning of ascorbic acid is not clear, but when the fact that browning of ascorbic acid occurs suddenly with a decrease in the reduced form of ascorbic acid due to auto oxidation is also considered, it seems that it acts on dehydroascorbic acid or on a metabolic pathway following diketogulonic acid to prevent the browning reaction.

Below, application examples will be given and effects will be explained.

Application Example 1

0.01 g of rutin was added to 1 g of ascorbic acid dissolved in 10 mL of ion exchanged water to give a suspension, which was held at 35°C shielded from light. The supernatant was filtered immediately after the experiment and again 10 days later, and light absorption by the visible part was measured. Material to which no rutin was added was used as a blank, and the difference in light absorption at 380 nm after 10 days was measured as ΔA380. The results are shown in Table 1.

Table 1

①	ΔA380
② ブランク	0.21
③ ルチン	0.10

Key: 1 Sample
2 Blank
3 Rutin

As is clear from Table 1, it can be seen that increased light absorption in the visible part of the ascorbic acid caused by browning is noticeably prevented by adding rutin.

Application Example 2

Flavonoid glycoside A or flavonoid glycoside B was dissolved to give a concentration of 0.1% in sodium ascorbate that had been adjusted to give 0.1% in 30% ethanol aqueous solution, and this was held at 25°C. Material with no flavonoid glycoside added was used as the blank, and the degree of browning, the ascorbic acid, and the dehydroascorbic acid (oxidized form of ascorbic acid) were measured after 1 week and after 4 weeks. For the degree of browning, in addition to observing with the naked dye, the L value was measured with a color-difference meter (model ND-504AA digital measurement color-difference meter made by Nippon Denshoku Industries Co. Ltd.). The L value corresponds to luminosity, and the greater the value, the higher the luminosity. Concerning the ascorbic acid, a high-speed liquid chromatograph was used to quantize using 265 nm light absorption. Concerning the dehydroascorbic acid, quantizing was performed suing a fluorescent measurement method using o-phenylenediamine.

The L value reduction percentage and the results of observation with the naked eye 4 weeks after the start of the experiment are shown in Table 2.

Table 2

	① サンプル	② L値減少率（%）	③ 肉眼による観察	
④	ブランク	100	かなり褐変して着色している	⑦
⑤	フラボノイド配糖体A	41.5	褐変はほとんど起きていない	⑧
⑥	フラボノイド配糖体B	89.5	褐変はほとんど起きていない	⑧

Key:
1 Sample
2 L value reduction percentage (%)
3 Observation with the naked eye
4 Blank
5 Flavonoid glycoside A
6 Flavonoid glycoside B
7 Considerable browning and discoloration
8 Hardly any browning occurred

The L value reduction percentage here is represented by the following formula.

$$\text{①減少率}(\%) = \frac{(\text{②試験開始時のアスコルビン酸NaのL値}) - (\text{③4週間後のサンプルのL値})}{(\text{②試験開始時のアスコルビン酸NaのL値}) - (\text{④4週間後のアスコルビン酸NaのL値})} \times 100$$

Key: 1 Reduction percentage
2 L value of sodium ascorbate immediately after preparation
3 L value of sample 4 weeks later
4 L value of sodium ascorbate 4 weeks later

The results of ascorbic acid measurement 1 week after the start of the experiment and measurements of ascorbic acid and dehydroascorbic acid after 4 weeks are shown in Table 3.

Table 3

1 サンプル	2 1週間後	4週間後 4	
	3 アスコルビン酸	アスコルビン酸	デヒドロアスコルビン酸 5
6 ブランク	26.0	trace	1.0
7 フラボノイド配糖体A	17.5	1.0	18.0
8 フラボノイド配糖体B	60.5	1.4	16.0

9 (単位：mg/100ml)

Key: 1 Sample
2 After 1 week
3 Ascorbic acid
4 After 4 weeks
5 Dehydroascorbic acid
6 Blank
7 Flavonoid glycoside A
8 Flavonoid glycoside B
9 Units

As is clear from Table 2, a noticeable ascorbic acid browning preventing effect was seen by flavonoid glycoside A or flavonoid glycoside B being present with sodium ascorbate. As is also clear from Table 3, comparing cases in which flavonoid glycoside A or flavonoid glycoside B is also present with ascorbic acid to the case in which they are not present, 10 times or more dehydroascorbic acid remains 4 weeks after the start of the experiment. Browning of ascorbic acid occurs suddenly with a decrease in the reduced form of ascorbic acid, and it seems that the browning reaction subsequent to dehydroascorbic acid is the cause of this. Therefore, it is thought that the fact that the reaction to the next step by dehydroascorbic acid is suppressed explains a part of the browning prevention effect of ascorbic acid by the flavonoid glycoside.

Effects of the invention

As is clear from the aforementioned application examples and experimental examples, the present invention relates to an ascorbic acid browning prevention method characterized in that a flavonoid glycoside is mixed in.